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Physicochemical Characterization and Antibacterial and Antifungal Activities of *Pistacia lentiscus* Oil in Northeastern Algeria

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ABSTRACT

The aim of this study is to validate the importance of *Pistacia lentiscus*, a species very widespread in northeastern Algeria, locally called "Dharou" as multipurpose pharmaceutical potential. Due to its richness in chemical components, it has been known for decades and widely used in the Arab and European pharmacopoeia in traditional medicine. In Algeria, this medicinal plant is mainly known and used in rural areas. Depending on the part of the plant, it is used to treat different diseases such as stomach ulcers, cough, diarrhea, bronchitis, burns, and eczema. To realize this study, we collected samples of lentisk oils traditionally extracted by the rural populations of seven regions, on which physicochemical analyzes (color, humidity, acidity index, peroxide index, phosphatide, saponification index) was carried out. The physicochemical parameters of oil collected were values corresponded to the international standards and generally reflecting a fairly oil's good quality, with the exception of the acidity index values, that exceeded standards in five regions. Exploring and evaluating their antibacterial and antifungal activities, using wells technique in order to determine the minimum inhibitory concentrations (MIC), were also done. The bacterial pathogens used were *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Kelbsiella pneumonia*. Meanwhile, for antifungal activity, the fungal strains tested were: *Verticillium sp*, *Pythium sp* and *Phytophthora sp*. All oils samples were ineffective against the bacterial strains tested. In contrary, antifungal activities were observed, suggesting that these oils can be used for biological control of fungi growth in various types of crops. Despite the exceptional medicinal virtues of *Pistacia lentiscus* in the Maghreb region, few studies have been devoted to this plant, which gives even more interest to our study.

Keywords: Antibacterial activity, Antifungal activity, Oil, *Pistacia lentiscus*, Physicochemical parameters.

INTRODUCTION

Algeria has a remarkable flora richness estimated at nearly 4000 taxa (Miara *et al.*, 2018). However, the medicinal flora has fading shadow with few studies on their importance (Baba Aissa, 2000; Hamel *et al.*, 2018). Among these medicinal plants listed, there is *Pistatia lentiscus*, an evergreen shrub of the Anacardiaceae family which is commonly dispersed in Algeria over the entire littoral. It is well known as "mastic tree" and has dark green leaves, with distinctive strong smell. The fruits are reddish-black berries when entirely ripen (Photo 1). From these edible ripen fruits is extracted an oil that was once commonly used in traditional medicine by rural populations. The phytochemical components of these fruits include tannins, essential oils, vitamins, flavonoids, etc. (Hamlat and Hassani, 2008).

Historically, the use of the plant and its oil made our ancestors know many of its virtues. Not so long ago, the branches and leaves of Lentisk were used to clean terracotta utensils. Nowadays, its oil is known for its many therapeutic benefits, especially on the respiratory tract and its aseptic properties. On both sides of the Mediterranean shores, traditional medicines attribute virtues to this plant in the treatment of ulcers, hypertension, cough, sore throat, eczema, kidney stones and jaundice (Gardeli *et al.*, 2008; Gonzalez-Tejero *et al.*, 2008; Ouelbani *et al.*, 2016; Bouasla and Bouasla, 2017, Senouci *et al.*, 2019).

Depending on the local region, *Pistacia lentiscus* fruit oil is often used as an external remedy applied locally as an ointment to treat burns (Bensegueni, 2007) or back pain (Bellakhdar, 1997). It is also used orally for respiratory problems of allergic origin and stomach ulcers (Miara *et al.*, 2018; Senouci *et al.*, 2019). In most of the rural localities where this plant grows, the production of its oil, which is based on purely traditional methods, is carried out by women (Djedaia, 2017; Lazli *et al.*, 2019; Senouci *et al.*, 2019). This undeniably lucrative activity has, however, opened up new horizons for other local populations, who have begun to produce at a more business-oriented production rate. Several actions have been carried out to increase the exploitation of *Pistacia lentiscus*, especially its fruit. The latter grows abundantly in the coastal forest scrubland, and a new effort is being made to boost the extraction of its oil by the inhabitants, due to the increase in the price per liter. Its extraction and crushing, which have remained at the stage of domestic craftsmanship, give a limited production for family use and can only be marketed in small quantities. Lentisk fruit oil is traditionally appreciated for its therapeutic and cosmetic uses and may be eligible for export or for use in specialized laboratories, which is a potential source of important income for farmers, especially rural women.

There are two methods of lentisk oil extraction: the traditional artisanal method and the mechanical method, the most recent. The first method is the oldest and the most widespread, it uses the stone grinder or on foot to grind the fruits. The decanting and separation of

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the oil is done in vats. The oil is collected after manual filtration (Camps and Faber, 1953). For the mechanical method, some modifications were made to the manufacturing process. Most of the basic operations were mechanized, with the exception of harvesting and washing, which remained manual. However, this mechanical extraction process is more practiced by men in the Lentisk oil mills.

Several studies have been carried out on *P. lentiscus*, using its different parts, to demonstrate its impressive uses and application. In 1999, Lanfranchi *et al.*, focused their study on the production of Lentisk oil in Sardinia. Alloune et al., (2012) carried their study on lentisk oil as a feedstock for biodiesel production. However, for phytochemical characterization of *P. lentiscus* extracted oil, Charef (2011) conducted the chemical composition and phytochemical and nutriational properties of oils extracted from the black and red fruits. Meanwhile, a study was carried out by Djerrou (2011) to explore the pharmacotoxicological effects of *P. lentiscus* vegetable oil. In parallel, Mezni *et al.*, (2012) evaluated the influence of the extraction method on the antioxidant and antibacterial activities of mastic fruit oil. Nevertheless, analysis of the fatty acid and sterol content of *P lentiscus* oil, during fruit ripening, was also studied by Trabelsi *et al*., (2012). Meanwhile, Dahmoune *et al*., (2014) investigated *P. lentiscus* leaves phenolic compounds and their extraction techniques. Moreover, Djerrou (2014) was conducted the anti-hypercholesterolemic activity of Lentisk vegetable oil. Concurrently, Maameri-Habibatni (2014) achieved a study which intended to provide a scientific basis for the use of *P. lentiscus* vegetable oil in traditional medicine. In 2015, Haouli *et al.*, carried out a study on the physicochemical and biochemical composition of the oil extracted from the plant fruits.

Given to the importance of *Pistacia lentiscus in* the socioeconomic life of the rural populations of the seven regions considered, this running study aims to improve the knowledge of its medicinal properties and its interest, through the characterization of the physicochemical parameters of the oils extracted from the fruits and the evaluation of their antibacterial and antifungal activities.

Photos (1): *Pistacia lentiscus* tree showing: A; woody shrub with evergreen leaves; B, pinnately compound leaves; C, reddish-black fruits (red circle).

MATERIALS AND METHODS

Source of the analyzed oil samples

The study was conducted in seven regions of eastern Algeria: Annaba, Skikda, Ain Khiar, Bougous, Bouhadjar, Guelma and Souk Ahras (Figure 1). The choice of these areas was based on the abundance, socio-economic interest and use of *Pistacia lentiscus* in traditional medicine. Lentisk oil samples were collected from local populations, from fully ripe fruits, after then oil was extracted according to the ancestral method. These samples were immediately placed in

hermetically sealed glass vials and wrapped in aluminum foil, and kept for laboratory analysis.

Lentisk oil physicochemical characteristics

The following physicochemical parameters were used to determine the quality of the oil samples considered. These parameters include: colour, humidity, acidity (free fatty acids formation because of rancidity), peroxide index (formation of primary oxidation products), phosphatide, saponification index and mucilage. They were determined in three replicates. The analyses were carried out at the "Society of fatty material" (*Société des Corps Gras)* Labelle Annaba, Spa (Algeria). These parameters were determined according to the methods mentioned below.

Color

The color of the collected Lentisk oil samples was determined using a colorimeter (Lovibond). Lovibond consists of three series of colored glasses: yellow, red and blue. Each series of glasses is additive, i.e. the absorbance of a given numbered glass is equivalent to the sum of the absorbance of two or more glasses, the sum of which is equal to that of the glass concerned. Color is an important factor in assessing the quality of oils. It reveals the nature of the technological treatment carried out on this oil. The color is indicated by mentioning the number of yellow, red and blue units, as follows:

$$C = xj + yr + pb$$

Where C: is the color of the oil; j: yellow pigment color, corresponding to chlorophyll; r: color pigment red, corresponding to carotenoid; b: blue color glass filter.

Humidity

It is the amount of water contained in a sample that is lost through heating. The humidity content is calculated from the difference in weight of a test sample before and after drying in an oven at 103°C ± 2° for 2±3 hours, as follows:

$$H\,\% = (m1 - m2)\,100/P$$

Where *P*, is the weight of the test sample; *m1*, is the oil mass in grams before drying; *m2*, is the oil mass in grams after desiccation.

Acidity index (AI %)

Acidity is the amount of free fatty acid resulting from the hydrolytic reactions of triglycerides. It is a quality criterion to report the state of conservation of oil (Kandji, 2001). It also allows controlling the level of hydrolytic, enzymatic or chemical degradation of triglyceride fatty acid chains (Abaza *et al.,* 2002).
The principle is based on a mixture of 10 mL of oil sample and 100 mL of ethyl-alcohol was heated until the content started boiling. The hot content was cooled and titrated with 15% KOH solution using phenolphthalein as endpoint indicator. The acid value is calculated as follows:

$$Acidity\ index\ (\%) = \frac{M.WtX\ V\ X\ N}{Pr}$$

Where *V*, is the number of milliliters of titrated solution of ethanolic KOH; *N*, is the exact normality of the standard solution of ethanolic KOH; *M.wt*, is the molecular weight of KOH (56.1g/mol) by whith molar mass 282 g /ml of oleic acid (specific to lentisk oil) (lentisk oil) and *Pr*, is the test sample in grams.

The peroxide index (PI)

It reflects the amount of peroxide present in the sample which is expressed in milliequivalents of active oxygen contained in one kilogram of product, oxidizing potassium iodide with iodine release. The PI index allows assessing the freshness of the oil, as follows:

$$Peroxide\ index\ (PI) = (V - V_0)\ x\ N\ x\ 100/M$$

Where *V*, is the volume of Na thiosulfate in the sample; *V0*, is the volume required to titrate the white; *N*, is the exact title of Na thiosulfate used and *M*, oil sample in grams.

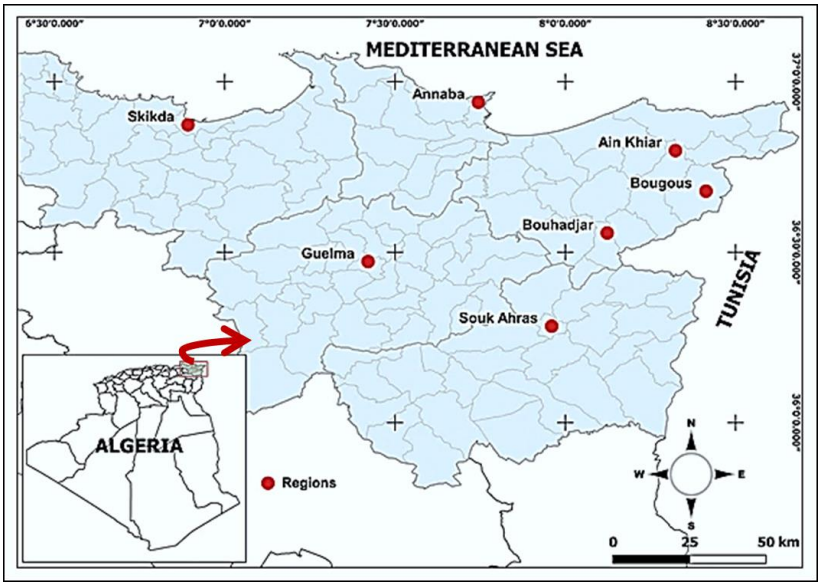

Figure (1): Location of provenance regions from where Lentisk oil samples were collected.

Phosphatides

Determination of phosphatides can be done by insolubilizing the phosphatides in a solvent of the oil that does not dissolve it like acetone, using the following equation:

$$\%\ Phosphatides = \frac{(P1 - P2)X100}{P}$$

Where *P1* and *P2*, are the filter weight before and after filtration; *P*, is the weight of the oil sample.

The saponification index (SI)

The saponification index is the number of milligrams of KOH required to neutralize the free acidity and to saponify the esters of 1 g of lipid. The value of the saponification index allows to estimate the lengths of the carbon chains of the fatty acids constituting the oil, and to calculate the average molecular weights of the fatty acids and triglycerides contained in the oil. The following equation was used for calculation:

$$SI = \frac{(V0 - V1)\ X\ 56.1\ X\ N}{P}$$

Where *SI*, is the Saponification index; *V0* and *V1*, are the volume of HCl solution used for the blank and oil sample, respectively; *N*, is Normality of the HCl solution and *P*, oil sample.

Determination of mucilage

A quantity of crude oil is poured into a beaker in a sand bath. When the temperature of the oil increases, a few drops of hydrochloric acid are added. The formation of a green ring is a sign of the existence of mucilage traces. The green coloring of all the oil will be a sign of the existence of a large quantity of mucilage.

Study of the antibacterial and antifungal properties

This part of the study was carried out in "Marzouk Ibrahim Polyclinic laboratory" at El-Tarf city. To evaluate the antibacterial activity of *Pistacia lentiscus* oil samples, Muller Hinton (MH) agar and B.G.T nutrient were used. Bacterial pathogens tested were propagated on nutrient broth to obtain a fresh culture. The bacterial strains tested were *Pseudomonas aeruginosa*, *Klebsiella pneumonia* (Gram-negative bacteria); and *Staphylococcus aureus* (Gram-positive bacteria).

The well technique was performed in which each well, 100 µl of oil samples (HV) were added on a bacterial seeded agar plate. After 24-hour incubation at 37°C, a reading was taken to detect the activity and determine the minimum inhibitory concentrations (MIC). Different concentration of plant extracts was prepared in test tubes by the two-in-two dilution method (1/2, 1/4, 1/8, 1/16, 1/32) from a stock solution with an initial concentration of 500 mg /ml (Ossou *et al.*, 2004). The activity of the samples was represented by a clear halo zone formed around each well. The results were expressed in four levels of activity following the method of Ponce *et al.*, (2003), in which (-) no activity; diameter of inhibition zone less than 8mm (ID < 8 mm) will consider (+); 9-14mm inhibition diameter will be given (++); strong activity of the samples will represent by (+++), ID is ranged between 15-19 mm (15mm ≤ D ≤ 19 mm), and very strong activity represents by (++++) in which ID is more than 20mm (D >20 mm).

For antifungal activity, work was performed in the Laboratory of Plant Biology at Chadli Benjedid University (El Tarf). Three fungal strains were tested: *Verticillium sp*, *Pythium sp* and *Phytophthora sp*. These fungal cultures are belong to group Oomycetes which are known for their pathogenic effect on plants as fruit trees, ornamental trees, vegetables, etc. PDA (Potato-Dextrose Agar) was used to carry the activity test.

Statistical analysis

The results were analyzed using statistical software R version 4.0.1 (2020). The data are represented in means ± standard deviation and $p< 0.05$ was considered as significance level. The analysis of variance (ANOVA One-way) and Tukey-test were performed to determine whether there was a difference or not in physicochemical parameters among Lentisk oil samples collected from different regions. Principal Component Analysis (PCA), using a matrix (Regions / oils physicochemical parameters) was also performed to evaluate and the rank Lentisk oil physicochemical variation among the studied regions.

RESULTS

Lentisk oil physicochemical characteristics

The average values of physicochemical parameters obtained from this study have shown highly differences between the seven regions from which our oil samples were collected (One-Way ANOVA, $p< 0.001$; Table 1).The yellow color parameter values at the different regions presented quite high values than red one. Furthermore, yellow color average values were higher than the International Olive Council (IOC) standards for the seven regions considered (Table 1). However, the red color average values were only above the IOC at five regions (Souk Ahras, Annaba, Ain Khiar, Bouhadjar and Bougous) (Table 1).

Humidity levels recorded for the different lentisk oil samples showed a variation in values from one locality to another but for the most part remain within the standards (≤ 1%). The percentages values were varied from 0.17% (Souk Ahras) to 0.99% (Guelma). Significant differences were recorded between humidity levels for the majority of oils tested (One-way Anova, p <0.000) (Table 1), except between: Guelma and Annaba (Tukey-test, p=0.99), Skikda and Souk Ahras (Tukey-test, p=0.71), Skikda and Ain Khiar (Tukey-test, p= 0.76) and Souk Ahras and Bouhadjar (Tukey-test, p= 0.09).

Acidity index of lentisk oils studied was above the standards for all localities except Skikda and Ain Khiar. The maximum value recorded was that of Bouhadjar (8.36%). High significant differences of

acidity levels were recorded between the majorities of oils tested (One-way Anova, $p < 0.000$) (Table 1). However, no significant difference was found between Bougous and Guelma oil samples (Tukey-test, p=0.99), Bouhadjar and Bougous (Tukey-test, p=0.69) and those of Bouhadjar and Guelma (Tukey-test, p= 0.91). The peroxide values recorded in this study were for the most part in compliance with standards. The highest significant value were those of Guelma oils (6.23 meq/kg), followed by oil samples collected from Skikda and Souk Ahras (4.18 meq/kg and 4.04 meq/kg, respectively). Meanwhile, the lowest value recorded was that of Bougous locality oil (0.54 meq/kg). Highly significant differences were verified among peroxide indices values of almost all lentisk oils tested (One-way Anova, $p < 0.000$) (Table 1). However, no significant differences were reported between: Skikda and Souk Ahras regions (Tukey-test, p= 0.96) and Ain Khiar and Skikda (Tukey-test, p= 0.74).

Saponification indices and phosphatide values showed values that remain within the international standards (IOC). The lowest saponification indices values were those of Bougous and Skikda, while, the highest but still within standards were those of Annaba and Guelma. For phosphatide values, the highest were recorded in oil samples collected from of Ain Khiar, followed by those of Guelma and Annaba. The analysis of variance showed high significant differences among oil samples collected from most regions (One-way Anova, $p < 0.000$) (Table 1). In addition, the mucilage test confirmed the purity of each oil sample.

Statistical interpretation of results

The Principal Component Analysis (PCA) revealed that the two first dimensions, representing 59% of the total variance, highlighted the difference among physicochemical parameters of region-oil samples considered in this study. The first dimension (38.5% of the total variance) separated regions with high AI (Bou-hadjar and Bougous) from the other regions (Figure 2). This is probably due to alteration of the oil composition during the extraction process, as an excess of water can combine with oil under local temperature which may lead to hydrolysis of triglycerides and an increase in acidity value (Figure 3; Table 1). The second axis (20.5% of the total variance) contrasted the regions with a highest values of physicochemical parameters indices (Peroxide index (PI), humidity (H), phosphatides (P) and saponification indices (SI) to lowest ones (Figures 2 and 3; Table 1).

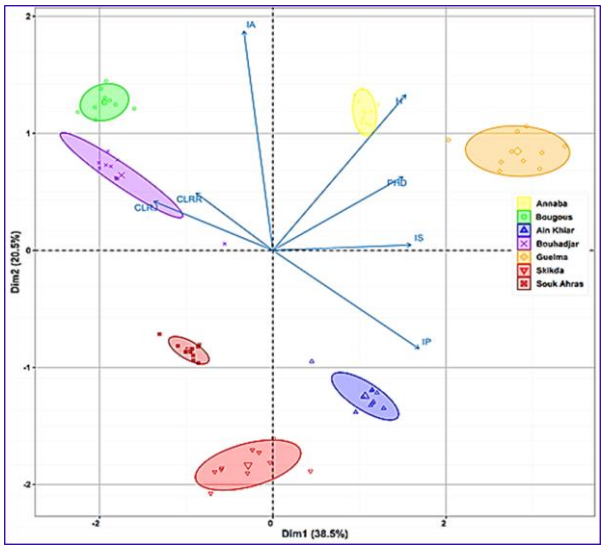

Figure (2): Principal correspondence analysis scatter plot with the first two components displaying the distribution of physico-chemical parameters considered in the study (ellipse= 95% confidence interval).

Study of the antibacterial and antifungal properties

Antibacterial activity

The oil samples tested showed no inhibitory effect on the three strains of bacteria tested. No inhibition zones were observed around the wells. Therefore, these samples have no antibacterial activity (S1).

Antifungal activity

Data obtained showed that Lentisk oil samples studied have an anti-fungal activity on fungal strains tested, where their activity was varied depending on the oil concentration (Table 2; S2).

Table (1): Physicochemical characteristics of the Lentisk oil samples. The values represented are means ± standard deviation.

Sampling regions	Measured Parameters							
	Color		Humidity (%)	Mucilage	Acidity index	Peroxide index ($meqO_2/Kg$)	Saponification index (mg)	Phosphatides (%)
	Yellow	Red						
Guelma	22.30 ±0.95	3.98±0.92	0.99 ±0.05	Brown	8.25 ±0.21	6.23 ±0.11	192.15 ±22.24	1.09 ± 0.08
Skikda	28.28 ±1.36	4.24 ± 0.53	0.21 ±0.06	Green	3.19 ±0.14	4.18 ±0.13	136.44 ±24.94	0.30 ± 0.03
Souk Ahras	55.67 ±0.39	9.49 ± 0.35	0.17 ±0.02	Brown	4.52 ±0.16	4.04 ±0.47	182.44 ± 0.19	0.20 ± 0.00
Annaba	38.30 ±1.49	8.50 ± 0.71	0.98 ±0.05	Green	5.72 ±0.26	2.82 ±0.12	193.4 ± 5.42	1.00 ± 0.00
Ain Khiar	21.84 ±0.50	8.08 ± 0.56	0.18 ±0.05	Green	2.24 ±0.12	3.95 ±0.73	178.30 ±1.89	1.31 ± 0.03
Bougous	34.38 ±0.36	10.16 ±0.63	0.33 ±0.08	Brown	8.22 ±0.22	0.54 ±0.13	117.76 ±0.35	0.69 ± 0.00
Bouhadjar	41.15±23.66	8.01 ± 1.89	0.23 ±0.09	Brown	8.36 ±0.27	2.27 ±0.07	144.52 ± 0.38	0.48 ± 0.00
IOC value[†]	**Y ≤ 7**	**R ≤ 8**	**≤ 1%**	-	**< 3.3 %**	**≤ 20**	**188 – 196 mg**	**184 – 196 mg**
Sign. level	**< 0.000*** **	**<0.000*** **	**<0.000*** **		**<0.000*** **	**<0.000*** **	**< 0.000*** **	**< 0.000*** **

[†]IOC, International Olive Council; Y: yellow color; R, red color; meq, milliequivalent; Kg, kilogram; mg: milligram

DISCUSSION

Few studies have been carried out on the physico-chemical quality and the antibacterial and antifungal power of medicinal plants oils. Most of researches conducted were devoted to ethnobotanical studies which have become increasingly important in recent decades, particularly in North Africa and Algeria (Fakchich and Elachouri, 2014; Boughrara and Legseir, 2016; Rhattas *et al*., 2016; Bouasla and Bouasla, 2017; Katiri *et al*., 2017; Miara *et al*., 2018; Lazli et al., 2019; Senouci *et al*., 2019; Zahir *et al*., 2020).

To evaluate Lentisk oils quality, the parameters selected in this study are considered as key of interest because they determine the shelf-life quality and hence the economic value of oils, which were confirmed by Decker et al., (2010) and Endo *et al*., (2018). Indeed, several authors have noted that rancidity of vegetable oils may pose health risks like cancer or inflammation because of the formation of toxic and reactive oxidation products (Mukherjee et al., 2009; Mehmood *et al*., 2012; Negash *et al*., 2019).

The "color parameter" was considered, in this study, to estimate the quality of oils where it has been proven that food pigments have beneficial effects on human health as antioxidant (Olson, 1999; Beutner *et al*., 2001; Moyano *et al*., 2010). Data obtained in the present study showed that oils color values were related to high levels of natural pigments in oil samples. This reported data are in agreement with data obtained, by Meléndez *et al*. (2003). These pigments are generally Chlorophyll and carotenoid pigments. They are important quality parameters because they correlate with color, which is a basic attribute of oil quality.

The importance of their content in the oil depends on various factors, including fruit maturity, climatic conditions, soil type and extraction methods (El Harfi *et al*., 2015). The results obtained indicate that the yellow color exhibited higher values than standards for the seven regions. On the other hand, the color red was above the standard values for five regions: Souk Ahras, Annaba, Ain Khiar, Bouhadjar and Bougous. In confirmation to our results Merzougui (2015) reported a value of 52 for the yellow color and 2 for the red color, in his study, on characterization of Lentisk oil in El Tarf, a locality in eastern Algeria not far from our study sites. The strong yellow coloring obtained in this study reflects very high levels of chlorophyll in all oil samples and particularly in Souk Ahras and Bougous regions, which also have high beta-carotene content, exceeding the standards. Several studies indicated that chlorophyll pigments are considered as antioxidants which have beneficial effect on human health (Lanfer-Marquez *et al*., 2005; Moyano *et al*., 2010) and may also be beneficial in the prevention of cancer (Ferruzzi and Blakeslee, 2007).

Some carotenoid are precursors of vitamin A and

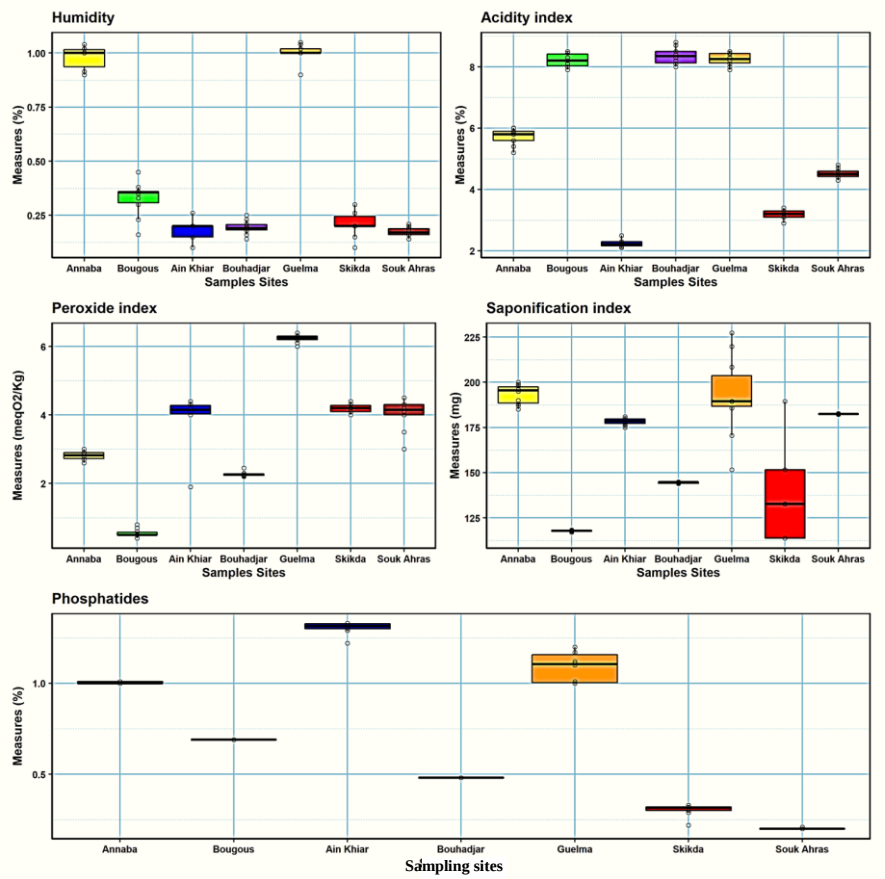

Figure (3): Variations in physicochemical parameters of oil samples collected from different study regions

Table (2): Antifungal activity of Lentisk oil samples, collected from different Algerian provenance regions and detection of the minimal inhibitory concentration on fungal pathogen tested.

Fungal strains	Guelma oil			
	Dilution used			
	T†	1/2	1/4	1/8
Phytophthora sp.	+	+	+	-
Verticillium sp.	+	+	+	+/-
Pythium sp.	+	+	+	-
	Skikda oil			
Phytophthora sp.	+	+	+/-	+
Verticillium sp.	+	+	+	+/-
Pythium sp.	+	+	+	+
	Souk Ahras oil			
Phytophthora sp.	+	+	+/-	-
Verticillium sp.	+	+	+	+/-
Pythium sp.	+	+	+/-	-
	Annaba oil			
Phytophthora sp.	+	+	+	+
Verticillium sp.	+	+	+	+
Pythium sp.	+	+	+	+
	Ain Khiar oil			
Phytophthora sp.	+	+	+	+
Verticillium sp.	+	+	+	+
Pythium sp.	+	+	+	+
	Bougous oil			
Phytophthora sp.	+	+	+	+/-
Verticillium sp.	+	+	+	+/-
Pythium sp.	+	+	+/-	-
	Bouhadjar oil			
Phytophthora sp.	+	+	+/-	-
Verticillium sp.	+	+	+/-	-
Pythium sp.	+	+	-	-

† T, 100 µl of oil used; (+), presence of inhibitory effect; (-), no antifungal activity.

effective antioxidants, they also prevent of serious human ailments like skin or cardiovascular diseases and as well cancer (Bernstein, 2002; Palozza, 2004; Voutilainen *et al.,* 2006; Wang *et al.,* 2008). They are natural chemical substances involved in the oxidation mechanisms of oil; their presence in sufficient quantities in the oil delays the photo-oxidation phenomenon and preserves the oil quality during storage (Lazzer *et al.,* 2006).

During this study, humidity levels recorded showed a variation in values from one locality to another but for the most part they remain within the standards. Several authors have reported various values compared with ours for the same regions. Bensalem (2015) reported a humidity of 0.64% for Skikda and 0.50% for Guelma. Merzougui (2015) reported that the humidity rate of 0.84% for El Tarf, which is higher than those obtained for Ain Khiar, Bougous and Bouhadjar, the three regions situated no far. This variation would be due to the artisanal extraction process, in which the use of water differs from one individual to another and probably from one region to another. To reduce humidity levels retained inside the fruits, these oils might require additional heating before extraction (Okechalu *et al.,* 2011; Negash *et al.,* 2019).

The oil humidity percentage is also a good indicator for hydrolysis of triglycerides (substance may pose a risk to human health). Indeed, high humidity percentage leads to the release of free fatty acids ten times more sensitive to oxidation than when they are in bound form. Higher humidity content, greater is the risk. Thus, it appears in our case that Guelma and Annaba oils are the most likely to present a risk of hydrolytic alteration and indicated that they are likely to undergo rancidity, which can drastically reduce their value by breaking down essential fatty acids (De Souza

et al., 2015; Negash *et al*., 2019).

The acidity index of Lentisk oils studied was higher than the standards for all localities except Skikda and Ain Khiar. Acidity controls the level of hydrolytic, enzymatic or chemical degradation of triglyceride fatty acid chains (Abaza *et al.,* 2002). Karleskind and Wolff (1992) indicated that a fat is protected against hydrolytic degradation if its acidity is ≤ 0.1%. The samples of Bouhadjar oil show the higher acidity value which is close to those noted by Maameri-Habibatni (2014) and Djerrou (2014), 8.5% and 7%, respectively for Lentisk oil from northeastern localities of Skikda and El Milia. The acidity index recorded for Skikda region in this study, 3.19%, is slightly higher than that reported by Boukeloua *et al.*, (2012), 2.27%, and Bensalem (2015) for Lentisk oil from Azzaba, located not far from Skikda. The latter values are practically similar to those obtained in this work for Ain Khiar locality, 2.24%. It can therefore be concluded that all the samples in this study may have been exposed to possible alterations. Demnati *et al.*, (2011) indicated that high acidity can be produced by the combined action of temperature and additional water during the artisanal mining process. Hilali *et al.*, (2005) reported that excess water catalyzed the hydrolysis of triglycerides and thus led to increased acidity. Another criterion to be considered is that the oils tested did not undergo any refining treatment in which soda neutralization is applied to them.

Most of the peroxide values recorded in this study are in compliance with standards. Other studies conducted with oils from the same regions indicate values more or less different. Merzougui (2015) and Bensalem (2015) reported values higher than those of this study, 5.39 meq/kg in El Tarf and 11.91 meq/kg in Skikda. However, for Guelma region, Bensalem (2015) obtained lower values than our study (4.17 meq/kg). The values obtained being less than 10 meq O_2/kg of oil, therefore, they comply with the standards of most conventional oils (FAO, 1981; ISO3960, 2007). Therefore, the oil samples studied can be considered to have an acceptable level of oxidation, as indicated by Rossell (1993). It demonstrates good hygienic and manufacturing practices for the oils sampled. Indeed, oxidation of oil begins after the fruit has been harvested from the tree, and continues during storage and processing. Fats can oxidize in the presence of oxygen and other factors like high temperature, water, enzyme, etc. (Chekroun, 2013). Good storage conditions and appropriate extraction methods used by artisans can help assess the early stages of oxidative deterioration of the product (Tchiegang *et al.,* 2004; Marmesat *et al.,* 2009). A high peroxide value could be induced by the presence of certain substances such as carotenoids, vitamins A and E, which can undergo similar oxidation reactions with peroxide formation (Kandji, 2001).

Saponification values calculated in this study showed values that remain within the standards. However, they were lower than those found in other researches, especially for the regions of Guelma and Skikda where Bensalem (2015) recorded an average value of 216.89 mg and 211.95 mg, respectively. Merzougui (2015) reported an average saponification index of 191.45 mg for El Tarf region, which is higher than the values obtained for oil samples of Ain Khiar, Bougous and Bouhadjar which are located no far.

Phosphatide values recorded, during this study, are for the most part in compliance with standards. The mucilage test confirmed the purity of the samples of tested oil. Thus, the green colored mucilages attest to be more or less pure oil, extracted under good conditions and free of impurities. This is the case of samples from Skikda, Annaba and Ain Khiar regions. The dark-colored mucilages (brown) probably indicate that during the extraction process the fruits were mixed with leaves, twigs or stems.

Highest values of humidity, phosphatides and saponification indices were depend on the pressing conditions and the quality of the oil: virgin (crude) or refined. The high saponification index reflects the low molecular weight of the fatty acids, which can be explained by the high moisture content that causes hydrolytic alteration of the oils. Therefore, differences in the values of the physicochemical parameters recorded for considered regions could be attributed to the variation in environmental factors and climate. Indeed, altitude is also factor modifying plant phytochemistry parameters and should be considered (Ibanez and Usubillaga, 2006 a&b; Tkachev *et al*., 2006; Haider *et al*., 2009).

The study of antibacterial and antifungal properties of Lentisk oils showed that antibacterial activity of the tested oil to specific bacterial pathogens used not effective (S1). These results may be related to bacterial strains used where they considered as resistant pathogens (Tassou and Nychas, 1995; Bonsignore *et al*., 1998; Benhammou and Bekkara, 2009). However, these oils exhibited an inhibitory effect against fungal strains tested where no fungal growth was recorded (S2). The fact that some bacterial strains are resistant to certain oils can be attributed to the data obtained. This prediction is consistent with those reported by Cosentino and Tuberoso (1999), De Billerbeck (2002) and Bammou *et al.*, (2015) who shown that extracts from other parts of *Pistacia lentiscus* (leaves, stem, mastic or gum) have antimicrobial effects on different types of strains. Indeed, a study reported by Tahiri (2008) showed that Lentisk leaves have a remarkable antimicrobial effect on *Salmonella enteritidis* with inhibition zones of 8.6±0.9; 16.5±1.3; 14.6±0.2 and 13.8±0.4 respectively at 5 µg/ml). In parallel, some literatures had reported that antibacterial activity of *Pistacia lentiscus* oil may be attributed to the chemical composition of the plant, including its richness in total polyphenols, flavonoids and tannins. Tannins have been shown to be bactericidal against several strains as: *Staphylococcus aureus* (Fogliani *et al*., 2005), *Bacillus cereus, Escherichia coli, Salmonella antum, Clostridium perfringins, Klebsiella pneumoniae* (Taguri *et al.,* 2006), *Vibrio parahaemolyticus* (Nagayama *et al.,* 2002), *Streptococcus bovis* (Jones *et*

al., 1994). Various studies have reported the high antioxidant capacity of the plant species (Longo *et al.,* 2007; Arab *et al.,* 2014; Dahmoune *et al.,* 2014; Djedaia 2017). Senouci *et al.,* (2019) reported that aerial parts contain terpineol, which is known to be effective against microbial activity. From the literature reviewed, it appears that *Pistacia lentiscus* leaves have a higher yield of phenolic compounds than fruits or essential oils (Arab *et al.,* 2014; Bampouli *et al.,* 2015).

In view of these mentions, it seems obvious that lentisk oil extracted from fruits does not have convincing effects with regard to bacterial strains compared to leaf extracts which are much richer in phenolic compounds. Due to the lack of antibacterial activity data on Lentisk oil samples tested in this study, it can be assumed that these oils may have low polyphenolic content. However, this should be verified in future researches. Bara *et al.,* (2007) reported the power of *Pistacia lentiscus* in inhibiting mycelial growth, which confirms the results of the antifungal activity obtained in this work with the fungal strains tested.

CONCLUSION

Although the results obtained in this study showed a variation in the physicochemical parameters among the several *Pistacia lentiscus* oils collected from different regions of north-eastern Algeria, their values remain within the standards attesting to a fairly good quality. However, the variations between these different physicochemical parameters can be explained by: (i) an alteration of oils during the extraction process, (ii) inappropriate storage conditions, (iii) variation in the environmental conditions of studied regions. The oils tested showed no antibacterial activity for the bacterial pathogen used but had an inhibitory effect on the fungal strains used.

These results are still preliminary and it would be interesting to conduct further studies to understand the molecular and cellular mechanisms of these effects. In addition, a phytochemical study should be carried out to determine the richness of the oil samples tested in phenolic compounds to better evaluate their antioxidant and antibacterial activities. Furthermore, the antifungal performances highlighted should be studied in more detail to consider the prospects for the application of these active ingredients as biological control agents capable of reducing the growth of fungi and moulds on field crops. Future work could also be envisaged to determine other biological activities of *Pistacia lentiscus (*anti-inflammatory, hypoglycaemic, anti-oxidant activities).

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الخصائص الفيزيائية والكيميائية والأنشطة المضادة للبكتريا والفطريات لزيت الفستق العدس في شمال شرق الجزائر

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الملخص العربـــــي

تهدف هذه الدراسة إلى المساهمة في تثمين واستغلال *Pistacia lentiscus* ،المسمى محليا بالضرو، وهي شجيرة تنمو في الأحراش في مناخات البحر الأبيض المتوسط وهي نوع واسع الانتشار في شمال شرق الجزائر. تنتمي هذه النبتة إلى عائلة *Anacardiaceae* .
نظرًا لغنائها بالمكونات الكيميائية التي تمنحها رائحة عطرية مميزة ، اشتهرت منذ عقود واستخدمت على نطاق واسع في مجلد الأدوية العربي والأوروبي في الطب التقليدي. في الجزائر ، يُعرف هذا النبات الطبي ويستخدم في المناطق الريفية ، حيث يحتفظ القدماء بالمعرفة. اعتمادًا على الجزء المستخدم من النبات ، يتم استخدامه لعلاج أمراض مختلفة مثل قرحة المعدة ، وارتفاع ضغط الدم ، والسعال ، والإسهال ، والتهاب الشعب الهوائية ، والحساسية الرئوية ، والحروق ، والأكزيما.. من أجل تنفيذ هذه الدراسة ، قمنا بجمع عينات من زيوت النبتة التي تم استخلاصها بشكل تقليدي من قبل سكان الريف في سبع مناطق ، حيث أجرينا تحليلات فيزيو كيميائية (اللون ، الرطوبة ، مؤشر الحموضة ، مؤشر البيروكسيد ، الفوسفاتيد ، مؤشر التصبن) و تم تقييم أنشطتهم المضادة للبكتيريا والفطريات .السلالات البكتيرية المختارة هي). (MICبتقنية الآبار لتحديد التركيزات المثبطة الدنيا *Pseudomonas aeruginosa* و *Klebsiella pneumonia* و *Staphylococcus aureus*
.بينما السلالاتالفطريه المختارة؛ *Verticillium sp* و *Pythium sp* و *Phytophthora sp*.
أظهرت النتائج أن قيم المعلمات الفيزيوالكيميائية للزيوت تتوافق مع المعايير ، باستثناء مؤشر الحموضة لخمسة مناطق ، مما يعكس بشكل عام جودة جيدة إلى حد ما. علاوة على ذلك ، كانت جميع عينات الزيوت غير فعالة ضد السلالات البكتيرية الثلاثة المختبرة. ومع ذلك ، فقد لوحظت أنشطة مضادة للفطريات المستخدمة ، مما يشير إلى أنه يمكن استخدام هذه الزيوت في المكافحة البيولوجية لنمو الفطريات في أنواع مختلفة من المحاصيل.
على الرغم من المزايا الاستثنائية المنسوبة إلى *Pistacia lentiscus* كنبات طبي في منطقة المغرب العربي ، فقد تم تخصيص القليل من الدراسات للممتلكات الفيزيائية والكيميائية والأنشطة المضادة للميكروبات ، مما يعطي اهتمامًا أكبر لدراستنا التي توفر بيانات أصلية ومثيرة للاهتمام يمكن تطويرها وتعميقها في المستقبل الأبحاث.